Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Fluoro Analog of UDP-α-D-Glucuronic Acid Is an Inhibitor of UDP-α-D-Apiose/UDP-α-D-Xylose Synthase

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Contents

S1. Materials and instrumentation

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further purification unless otherwise specified. *Escherichia coli* DH5α cells were obtained from Bethesda Research Laboratories (Muskegon, MI). The vector pET28b(+) and enzyme KOD DNA polymerase were purchased from Novagen (Madison, WI). DNA modifying enzymes (for restriction digestion and ligation), PCR primers, and the overexpression host *E. coli* BL21 star (DE3) were acquired from Invitrogen (Carlsbad, CA) and New England Biolabs (NEB, Beverly, MA). LB media were products of Difco (Detroit, MI) or Fisher Scientific, and pre-stained protein markers were the product of NEB. Ni-NTA agarose and kits for DNA gel extraction and spin miniprep were obtained from Qiagen (Valencia, CA). All reagents for SDS-PAGE and Amicon YM-10 filtration products were purchased from Bio-Rad (Hercules, CA) and Millipore (Billerica, MA), respectively. Analytical thin layer chromatography (TLC) was carried out on precoated TLC glass plates (Silica gel, grade 60, F254, 0.25mm layer thickness) obtained from EMD chemicals (Madison, WI). Flash column chromatography was performed on silica gel (230-400 mesh, grade 60) from Sorbent Technologies (Atlanta, GA) by elution with the specified solvents. Protein concentrations were determined by Bradford assay using bovine serum albumin as the standard. The relative molecular mass and purity of enzyme samples were determined using SDS-polyacrylamide gel electrophoresis as described. The general methods and protocols for recombinant DNA manipulations were as described by Sambrook and Russell[1]. DNA sequencing was performed by the Core Facilities in the Institute of Cellular and Molecular Biology of the University of Texas at Austin. The NMR spectra

were acquired on a Varian Unity Inova 500 or 600 MHz spectrometer housed in the NMR Facility of the Department of Chemistry and Biochemistry, University of Texas at Austin. The MS analyses were carried out at the Mass Spectrometry and Proteomics Facility of the Department of Chemistry and Biochemistry, University of Texas at Austin.

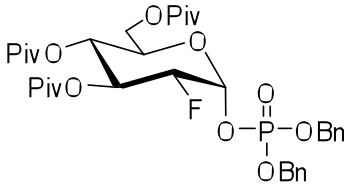
S2. Preparation of AXS and UDP-2F-GlcA (9)

S2.1. Preparation of AXS

Gene *axs1* was identified as the coding gene for UDP-D-apiose synthase in *Arabidopsis*.[2] Based on this information, RNA was extracted from the leaves of *Arabidopsis*, reverse transcribed into cDNA, the gene *axs1* encoding UDP-apiose/UDP-xylose synthase was amplified, and cloned into the pET28b(+) vector. The *Escherichia coli* BL21 (DE3) cells transformed with this recombinant plasmid expressed very little soluble UDP-apiose synthase protein. The *axs1* gene was further cloned into a maltose binding protein (MBP) fusion vector (MalE-pET)[3] at *NdeI* and *EcoRI* sites after a silent mutation at nucleotide 552 of the *axs1* gene to eliminate an internal *NdeI* site. The resulting plasmid was then transformed into *Escherichia coli* BL21 (DE3) cells. The cells were grown at 37 °C in 6 L of LB broth containing kanamycin (50 μg/mL) until the OD600 reached approximately 1.0. Expression of the MBP-apiose synthase fusion protein was induced by the addition of 50 μM IPTG. The cells were cultured for an additional 18 h at 15 °C, harvested by centrifugation and stored at −80 °C. Thawed cells were resuspended in lysis buffer (50 mM Tris buffer (pH 8.0), 1 M NaCl, 10 mM imidazloe, 10% glycerol and 1 mM β-mercaptoethanol) and sonicated to lyse the cells. After centrifugation at 25,000 × *g* for 20 min, the lysate was incubated with 10 mL Ni-NTA agarose (Qiagen) for 1 to 2 h prior to extensive washing with washing buffer (50 mM Tris buffer (pH 8.0), 1 M NaCl, 20 mM imidazloe, 10% glycerol and 1 mM β-mercaptoethanol). The MBP-AXS fusion protein was eluted from the Ni-NTA agarose with a buffer similar to that described above containing 250 mM imidazole, spiked with 5% (w/w) His_6-tagged TEV protease to cleave off the His_{10}-MBP fragment from the fusion protein, and dialyzed against lysis buffer at 4 °C for 1 to 2 days with 2 changes of lysis buffer. The unwanted His_{10}-MBP and His_6-TEV was removed by passing the lysate through a 10 mL Ni-NTA column. The untagged AXS was then concentrated and dialyzed against 50 mM Tris buffer (pH 8.0), 300 M NaCl, 10% glycerol and 1 mM β-mercaptoethanol prior to use.

S2.2. Preparation of UDP-2F-GlcA

Synthesis of compound 19



To a flask containing compound **18**[4] (α/β mixture, 14.89 g, 34.3 mmol), *1H*-tetrazole solution in acetonitrile (0.45 M, 114 mL, 51.3 mmol) was added, and the mixture was concentrated *in vacuo* and dried under high vacuum for 2 h. Anhydrous CH_2Cl_2 (300 mL) was added, the reaction mixture was cooled to –20 °C, and dibenzyl *N,N*-diisopropylphosphoramidite (12.3 mL, 51.4 mmol) was added dropwise over 15 min. The reaction was stirred for 2 h at –

20 °C and then warmed to room temperature. After stirring for 4 h at room temperature, the reaction mixture was cooled to –40 °C and *m*CPBA (41.0 g, 60% purity, 120 mmol) was added portionwise over 30 min. After stirring for 3 h at –40 °C, the reaction mixture was warmed to room temperature and stirred for an additional 5 h. A saturated, aqueous solution of $NaHCO_3$ (300 mL) was slowly added, and the reaction mixture was extracted with CH_2Cl_2 (200 mL × 2). The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford compound **19** (14.52 g, 20.9 mmol, 61%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35 – 7.32 (m, 10H, Ph), 5.95 (dd, 1H, *J* = 3.7, 6.8 Hz, 1-CH), 5.54 (ddd, 1H, *J* = 9.65, 9.64, 11.5 Hz, 3-CH), 5.09 (d, 2H, *J* = 7.6, CH_2 of Bn), 5.08 (d, 1H, *J* = 8.8 Hz, CH_2 of Bn), 5.11 – 5.05 (m, 1H, 4-CH), 4.55 (dddd, 1H, *J* = 2.8, 3.5, 9.6, 48.8 Hz, 2-CH), 3.99 – 3.89 (m, 2H, 5-CH + 6-CH_2), 1.17 (s, 9H, CH_3 of Piv), 1.16 (s, 9H, CH_3 of Piv), 1.13 (s, 9H, CH_3 of Piv).

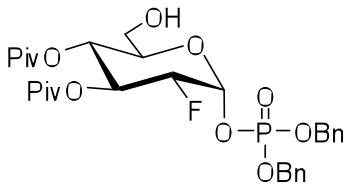
^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.79 (C=O of Piv), 177.07 (C=O of Piv), 176.22 (C=O of Piv), 135.38 (d, *J* = 6.4 Hz, Ph-C), 135.29 (d, *J* = 7.4 Hz, Ph-C), 128.71 (Ph), 128.67 (Ph), 128.64 (Ph), 128.61 (Ph), 128.16 (Ph), 127.93 (Ph), 93.80 (dd, *J* = 5.5, 22.1 Hz, 1-CH), 87.21 (dd, *J* = 7.8, 196.5 Hz, 2-CH), 69.87 – 69.50 (m, 5-CH, 2 x CH_2 of Bn, + 3-CH), 66.15 (d, *J* = 6.9 Hz, 4-CH), 60.77 (6-CH_2), 38.81 (C of Piv), 38.79 (C of Piv), 38.72 (C of Piv), 27.03 (CH_3 of Piv), 27.02 (CH_3 of Piv), 26.99 (CH_3 of Piv).

^{19}F NMR ($CDCl_3$, 564 MHz) δ –201.3 (dd, *J* = 11.6, 48.8 Hz).

^{31}P NMR ($CDCl_3$, 242 MHz) δ –2.77.

HRMS (ESI, positive mode) Calcd for $C_{35}H_{48}O_{11}FNaP^+$ 717.2811, Found 717.2812.

Synthesis of compound 20



To an anhydrous CH_2Cl_2 solution of compound **19** (7.35 g, 10.6 mmol), DIBAL-H (1M in CH_2Cl_2, 12.7 mL) was added dropwise over 40 min at –78 °C. The reaction was stirred for 4 h at that temperature and quenched by addition of a saturated, aqueous solution of NH_4Cl (100 mL). The reaction mixture was stirred for an additional 30 min at room temperature and filtered using a celite pad. The filtrate was extracted with CH_2Cl_2 (150 mL x 2). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford compound **20** (2.76 g, 4.52 mmol, 40%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 – 7.31 (m, 10H, Ph), 5.95 (dd, 1H, *J* = 3.7, 6.4 Hz, 1-CH), 5.62 (ddd, 1H, *J* = 9.58, 9.64, 11.9 Hz, 3-CH), 5.09 (d, 2H, *J* = 7.7, CH_2 of Bn), 5.086 (d, 1H, *J* = 8.6 Hz, CH_2 of Bn), 5.01 (dd, 1H, *J* = 9.89, 9.94 Hz, 4-CH), 4.54 (dddd, 1H, *J* = 2.9, 3.6, 9.5, 48.7 Hz, 2-CH), 3.81 (ddd, 1H, *J* = 2.1, 3.5, 10.3 Hz, 5-CH), 3.49 (dd, 1H, *J* = 2.1, 13.1 Hz, 6-CH_2), 3.35 (dd, 1H, *J* = 3.9, 13.1 Hz, 6-CH_2), 1.17 (s, 9H, CH_3 of Piv), 1.15 (s, 9H, CH_3 of Piv).

^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.93 (C=O of Piv), 177.00 (C=O of Piv), 135.40 (d, *J* = 7.0 Hz, Ph-C), 135.27 (d, *J* = 7.5 Hz, Ph-C), 128.72 (Ph), 128.70 (Ph), 128.64 (Ph), 128.17 (Ph), 127.96 (Ph), 93.80 (dd, *J* = 5.0, 21.9 Hz, 1-CH),

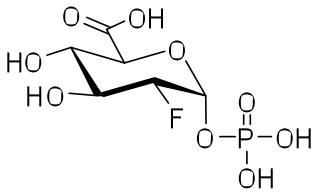
87.27 (dd, J = 8.5, 196.5 Hz, 2-CH), 71.71 (5-CH), 69.92 (d, J = 5.5 Hz, CH_2 of Bn), 69.732 (d, J = 5.3 Hz, CH_2 of Bn), 69.11(d, J = 19.4 Hz, 3-CH), 67.04 (d, J = 7.5 Hz, 4-CH), 60.34 (6-CH_2), 38.89 (C of Piv), 38.84 (C of Piv), 27.05 (CH_3 of Piv), 27.02 (CH_3 of Piv).

^{19}F NMR ($CDCl_3$, 470 MHz) δ –200.74 (dd, J = 12.0, 48.7 Hz).

^{31}P NMR ($CDCl_3$, 202 MHz) δ –2.03.

HRMS (ESI, positive mode) Calcd for $C_{30}H_{40}O_{10}FNaP^+$ 633.2235, Found: 633.2235.

Synthesis of compound 23



To CrO_3 (1.28 g, 12.8 mmol) in H_2O (20 mL), conc. H_2SO_4 (1.2 mL, 21.6 mmol) was added dropwise over 5 min at 0 °C. The CrO_3/H_2SO_4 mixture was added to a solution of compound **19** (1.40 g, 2.29 mmol) in acetone (80 mL) at 0 °C, and the reaction mixture was stirred overnight while allowing the temperature to rise to room temperature. The reaction mixture was then evaporated to remove the acetone and extracted with $CHCl_3$ (50 mL × 7). The combined $CHCl_3$ layers were dried over Na_2SO_4 and concentrated. The crude compound **21** was dissolved in MeOH (70 mL) and 10 % Pd/C (300 mg) was added and stirred under a hydrogen atmosphere (1 atm) for 3 h. The reaction mixture was filtered using a celite pad and washed with MeOH (30 mL). The resulting filtrate was concentrated *in vacuo* and purified by DEAE ion exchange resin column chromatography (6 × 25 cm, total 2 L of 0 ~ 0.5 M NH_4HCO_3 gradient). The fractions containing the product were collected and concentrated *in vacuo*. To the residue was added 30 mL of water and lyophilized (× 2) to give the compound **22** (340 mg, 0.77 mmol, 33%).

Compound **22** (220 mg, 0.50 mmol) was dissolved in anhydrous MeOH (30 mL), cooled to 0 °C, and treated with a 4.37 M solution of NaOMe in MeOH (1.6 mL, 7.0 mmol). After 7 h at room temperature, the reaction mixture was concentrated and purified by DEAE ion exchange resin column chromatography (2.5 × 20 cm, total 1 L of 0 ~ 0.5 M NH_4HCO_3 gradient). The fractions containing the product were collected and concentrated *in vacuo*. To the residue was added 25 mL of water and lyophilized (× 2) to give compound **23** (119 mg, 0.43 mmol, 86%). In order to change the salt form, compound **23** (110 mg, 3.98 mmol) was dissolved in 1:1 mixture of 10 mL of water and triethylamine. After stirring overnight at room temperature, the reaction mixture was concentrated to afford 151 mg of TEA salt of compound **22** (1.63 eq. TEA salt).

^{1}H NMR (D_2O, 600 MHz) δ 5.57 (dd, 1H, J = 3.6, 7.8 Hz, 1-CH), 4.35 (dddd, 1H, J = 2.2, 3.7, 8.8, 43.7 Hz, 2-CH), 4.03 (d, 1H, J = 10.2 Hz, 5-CH), 3.91 (ddd, 1H, J = 9.39, 9.39, 12.9 Hz, 3-CH), 3.46 (dd, 1H, J = 9.7, 9.8 Hz, 4-CH).

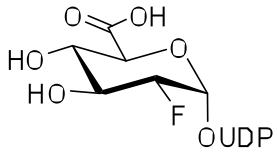
^{13}C NMR (D_2O, 150 MHz) δ 175.26 (6-C=O), 91.77 (dd, J = 5.2, 22.5 Hz, 1-CH), 89.29 (dd, J = 7.7, 188.0 Hz, 2-CH), 72.54 (5-CH), 71.38 (d, J = 8.0 Hz, 4-CH), 70.78 (d, J = 17.9 Hz, 3-CH).

^{19}F NMR (D_2O, 564 MHz) δ –200.16 (dd, J = 12.6, 49.5 Hz).

^{31}P NMR (D_2O, 242 MHz) δ –1.40.

HRMS (ESI, negative mode) Calcd for $C_6H_9FO_9P^-$ 274.9974, Found: 274.9976.

Synthesis of compound 9



To the TEA salt of compound **23** (105 mg, 0.277 mmol) in dry pyridine (10 mL) were added 1H tetrazole (50 mg, 0.710 mmol) and UMP-morpholidate (247 mg, 0.360 mmol). After 3 days at room temperature, the reaction mixture was concentrated *in vacuo*. Diethyl ether (10 mL) and H_2O (10 mL) were added to the residue, and the water layer was concentrated and purified by DEAE ion exchange resin column chromatography (2.5 × 20 cm, total 1 L of 0 ~ 0.4 M NH_4HCO_3 gradient). Fractions containing the product were collected and dried *in vacuo*, and lyophilized (× 2) to afford the compound **9** (35 mg, 0.0584 mmol, 21 %).

^{1}H NMR (D_2O, 600 MHz) δ 7.85 (d, 1H, *J* = 8.1 Hz, 6-CH), 5.88 (d, 1H, *J* = 4.8 Hz, 1′-CH), 5.86 (d, 1H, *J* = 8.1 Hz, 5-CH), 5.69 (dd, 1H, *J* = 3.5, 11.4 Hz, 1″-CH), 4.37 (dddd, 1H, *J* = 2.4, 3.5, 9.5, 49.1 Hz, 2″-CH), 4.26 – 4.23 (m, 2H, 2′-CH + 3′-CH), 4.17 – 4.14 (m, 1H, 4′-CH), 4.12 – 4.03 (m, 2H, 5′-CH_2), 4.06 (d, 1H, J = 10.1 Hz, 5″-CH), 3.91 (ddd, 1H, *J* = 9.4, 9.4, 12.8 Hz, 3″-CH), 3.44 (dd, 1H, *J* = 9.6, 9.9 Hz, 4″-CH).

^{13}C NMR (D_2O, 150 MHz) δ 175.72 (6″-C=O), 166.19 (4-C=O), 151.83 (2-C=O), 141.61 (6-CH), 102.69 (5-CH), 92.31 (dd, *J* = 5.5, 22.4 Hz, 1″-CH), 89.19 (dd, *J* = 8.0, 188.2 Hz, 2″-CH), 88.12 (1′-CH), 83.39 (d, *J* = 9.2 Hz, 4′-CH), 73.73 (2′-CH), 72.79 (5″-CH), 71.37 (d, *J* = 8.0 Hz, 4″-CH), 70.72 (d, *J* = 17.9 Hz, 3″-CH), 69.72 (3′-CH), 64.90 (d, *J* = 5.5 Hz, 5′-CH_2).

^{19}F NMR (D_2O, 564 MHz) δ –200.73 (dd, *J* = 12.8, 49.1 Hz).

^{31}P NMR (D_2O, 242 MHz) δ –1.54 (d, *J* = 17.9 Hz), –13.39 (d, *J* = 17.9 Hz).

HRMS (ESI, negative mode) Calcd for $C_{15}H_{20}FN_2O_{17}P_2^-$ 581.0227, Found: 581.0228.

S3. *In vitro* enzyme activity assays

S3.1. Activity assay of AXS using compound 9 as a substrate

To a solution containing compound **9** (400 μM), and NAD^+ (400 μM) in 50 mM Tris buffer (pH 8.0) at room temperature was added AXS (10 μM) to initiate the enzyme reaction (total volume 250 μL). The reaction mixture was incubated for 40 h at which time a 40 μL aliquot of the reaction mixture was taken and filtered using a YM10 microcentrifugal filter in order to remove AXS. The filtrate (20 μL) was subjected to HPLC analysis using a Dionex CarboPacTM PA1 4 × 250 mm column. The column was eluted using a linear gradient of 5 – 30% of solvent B over 40 min and then 30 – 100% of solvent B over another 5 min (flow rate; 1 mL/min, solvent A: H_2O, solvent B: 1M NH_4OAc). The result is shown in **Figure S1**.

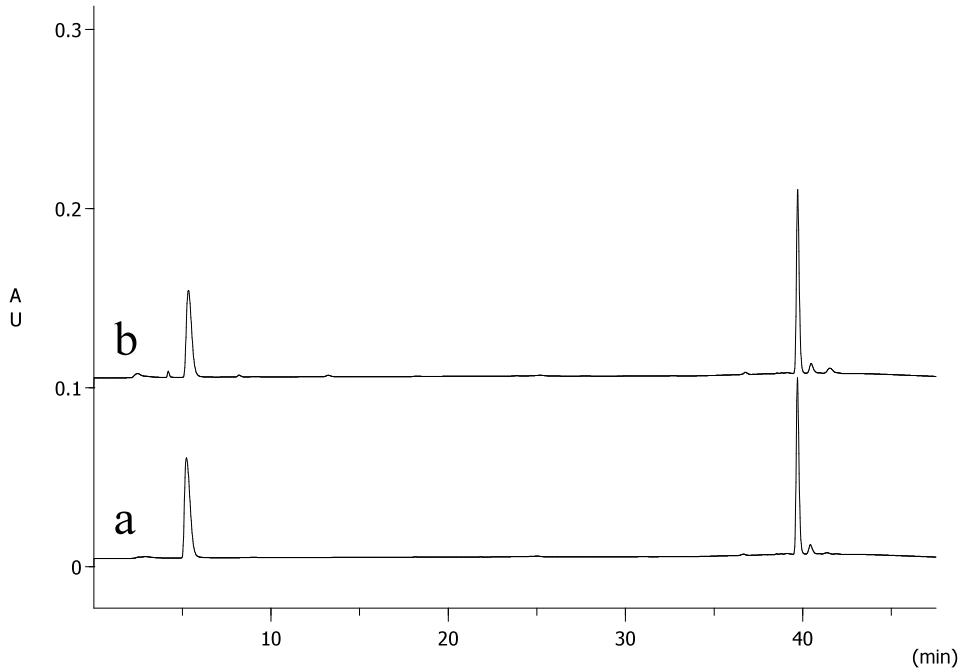


Figure S1. HPLC traces of AXS reaction. The reaction mixture containing 10 μM AXS, 400 μM NAD^+ and 400 μM UDP-2F-GlcA (**9**) in 50 mM Tris buffer (pH 8.0) was incubated for (a) 0 h, (b) 40 h.

S3.2. AXS inhibition assay using compound 9 as an inhibitor

The reaction mixtures containing AXS (10 μM), NAD^+ (400 μM), UDP-GlcA (**3**) (400 μM) and different concentrations of **9** (0, 400 μM, or 4 mM) in 50 mM Tris buffer (pH 8.0) (total volume 250 μL) were incubated at room temperature. At each given time point (0, 2, 6, 10, 14, and 20 h), reaction aliquots were analyzed by HPLC as described above. The results are shown in **Figure S2** – **S4**.

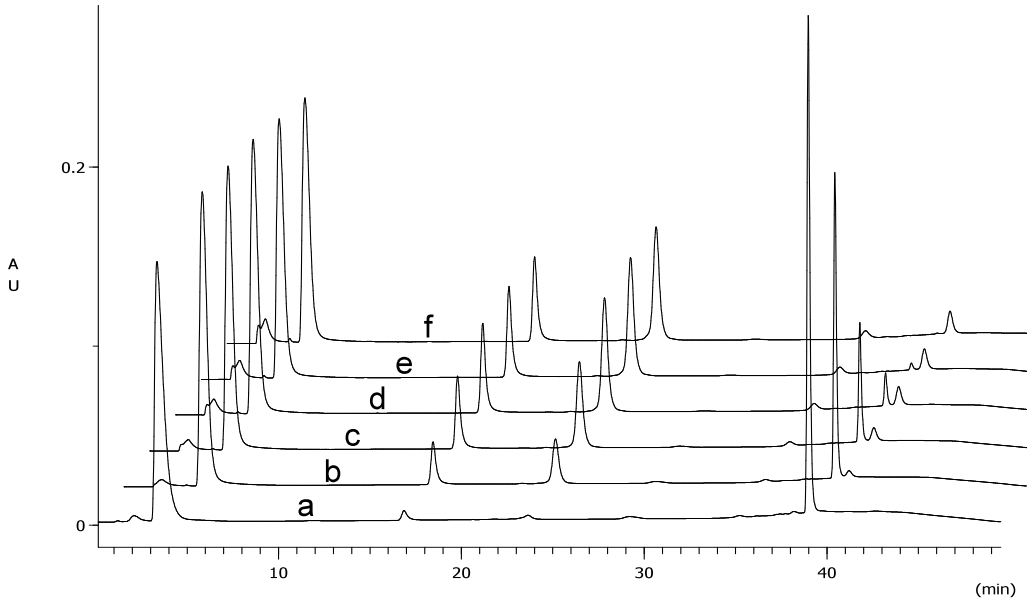


Figure S2. HPLC traces of AXS reaction. The incubation mixture containing 10 μM AXS, 400 μM NAD^+ and 400 μM UDP-GlcA (**3**) in 50 mM Tris buffer (pH 8.0) was incubated for (a) 0 h, (b) 2 h, (c) 6 h, (d) 10 h, (e) 14 h and (f) 20 h.

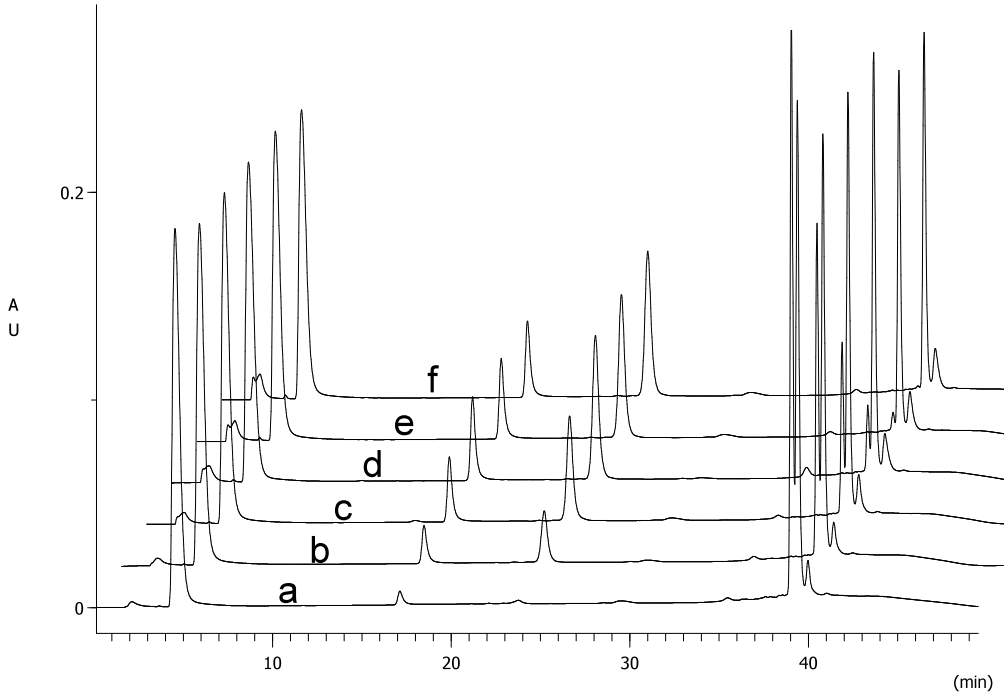


Figure S3. HPLC traces of AXS reaction. The incubation mixture containing 10 μM AXS, 400 μM NAD^+, 400 μM UDP-GlcA (**3**) and 400 μM UDP-2F-GlcA (**9**) in 50 mM Tris buffer (pH 8.0) was incubated for (a) 0 h, (b) 2 h, (c) 6 h, (d) 10 h, (e) 14 h and (f) 20 h.

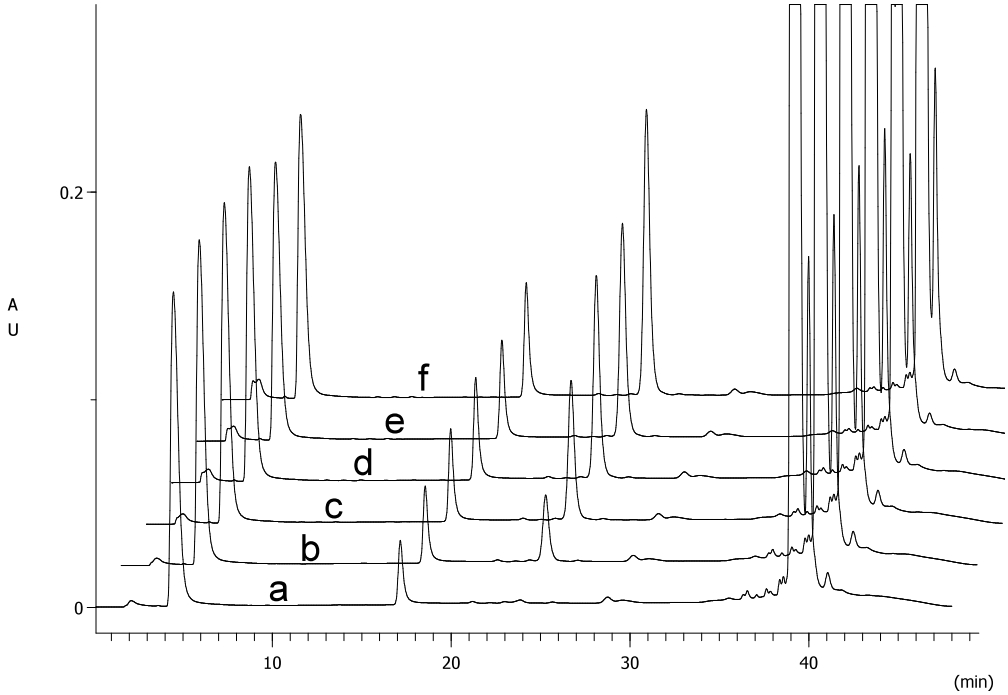


Figure S4. HPLC traces of AXS reaction. The incubation mixture containing 10 μM AXS, 400 μM NAD^+, 400 μM UDP-GlcA (**3**) and 4 mM UDP-2F-GlcA (**9**) in 50 mM Tris buffer (pH 8.0) was incubated for (a) 0 h, (b) 2 h, (c) 6 h, (d) 10 h, (e) 14 h and (f) 20 h.

S3.3. Examining the stability of UDP-GlcA (3), UDP-xylose (8), and UDP-2F-GlcA (9)

Individual reaction mixtures containing 400 μM of **3**, **8**, or **9** in 50 mM Tris buffer (pH 8.0) were incubated for 44 h, respectively (total volume 100 μL). At each time point (0 h, 20 h, and 44 h), 20 μL of the each reaction mixture was subjected to HPLC analysis as described above. The results are shown in **Figure S5**.

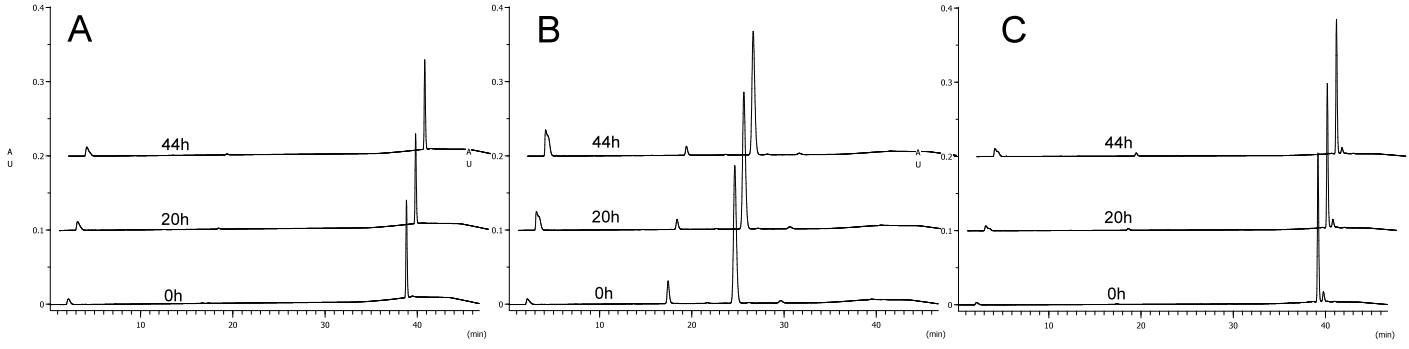


Figure S5. HPLC trace showing the stability of **3**, **8**, and **9**. The reaction mixtures containing 400 μM of (A) UDP-GlcA (**3**), (B) UDP-xylose (**8**), or (C) UDP-2F-GlcA (**9**) were incubated in 50 mM Tris buffer (pH 8.0) for 44 h.

S3.4. Structural characterization of the oxime derivative of UDP-4-KX

A reaction mixture containing 10 μM AXS, 400 μM NAD^+, and 400 μM UDP-GlcA (**3**) in 50 mM Tris buffer (pH 8.0) was incubated for 12 h. An aliquot of the reaction mixture was then subjected to HPLC analysis as described above. A second 40 μL aliquot of the reaction mixture was mixed with 40 μL of 400 mM H_2NOH in water. The mixture was filtered using a YM10 centrifugal filter as described above and incubated for an additional 3 h before being subjected to HPLC analysis (**Figure 1**). The peak at 35 min was collected, lyophilized, and subjected to MS analysis. The result is shown in **Figure S6**.

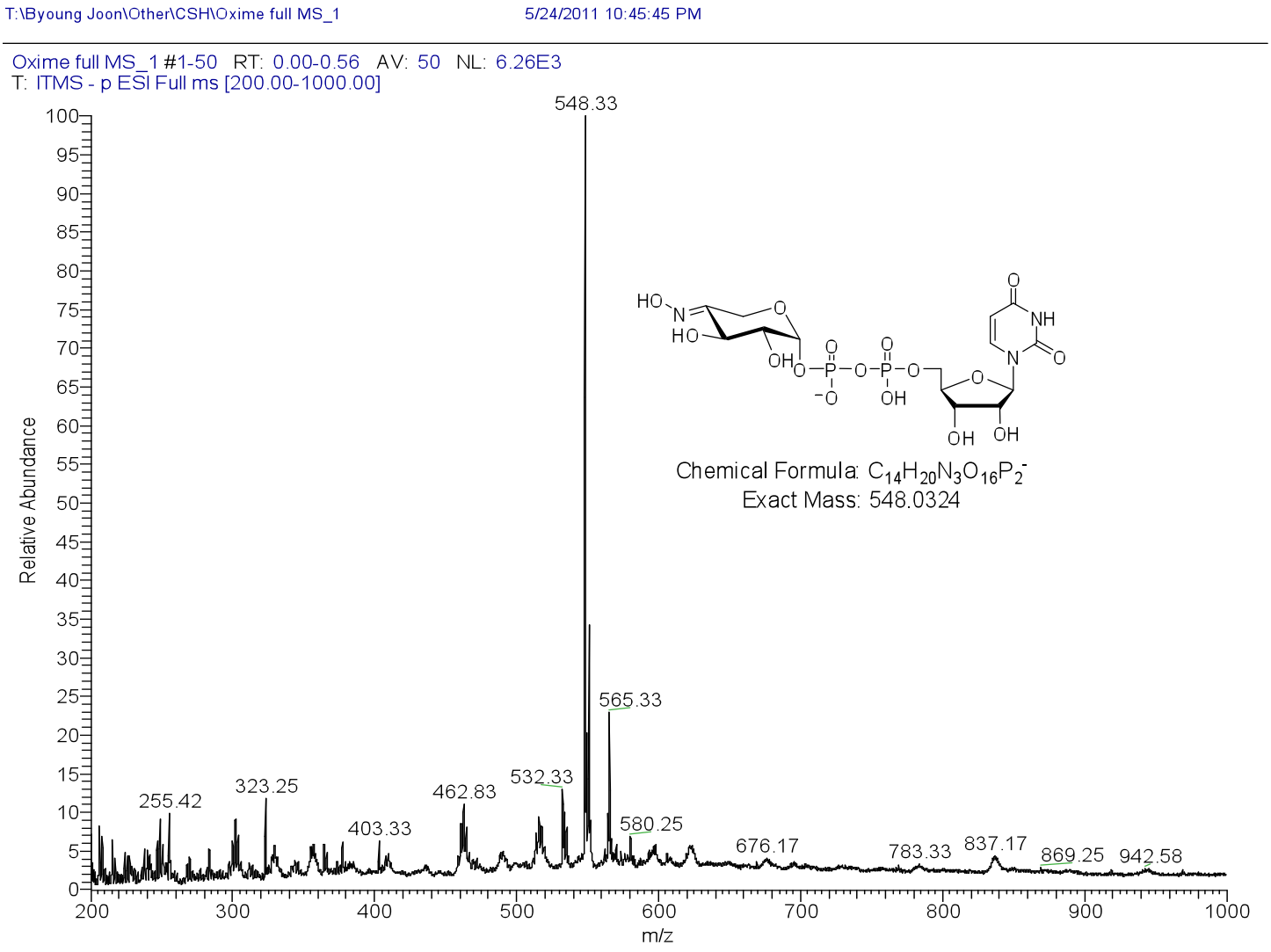
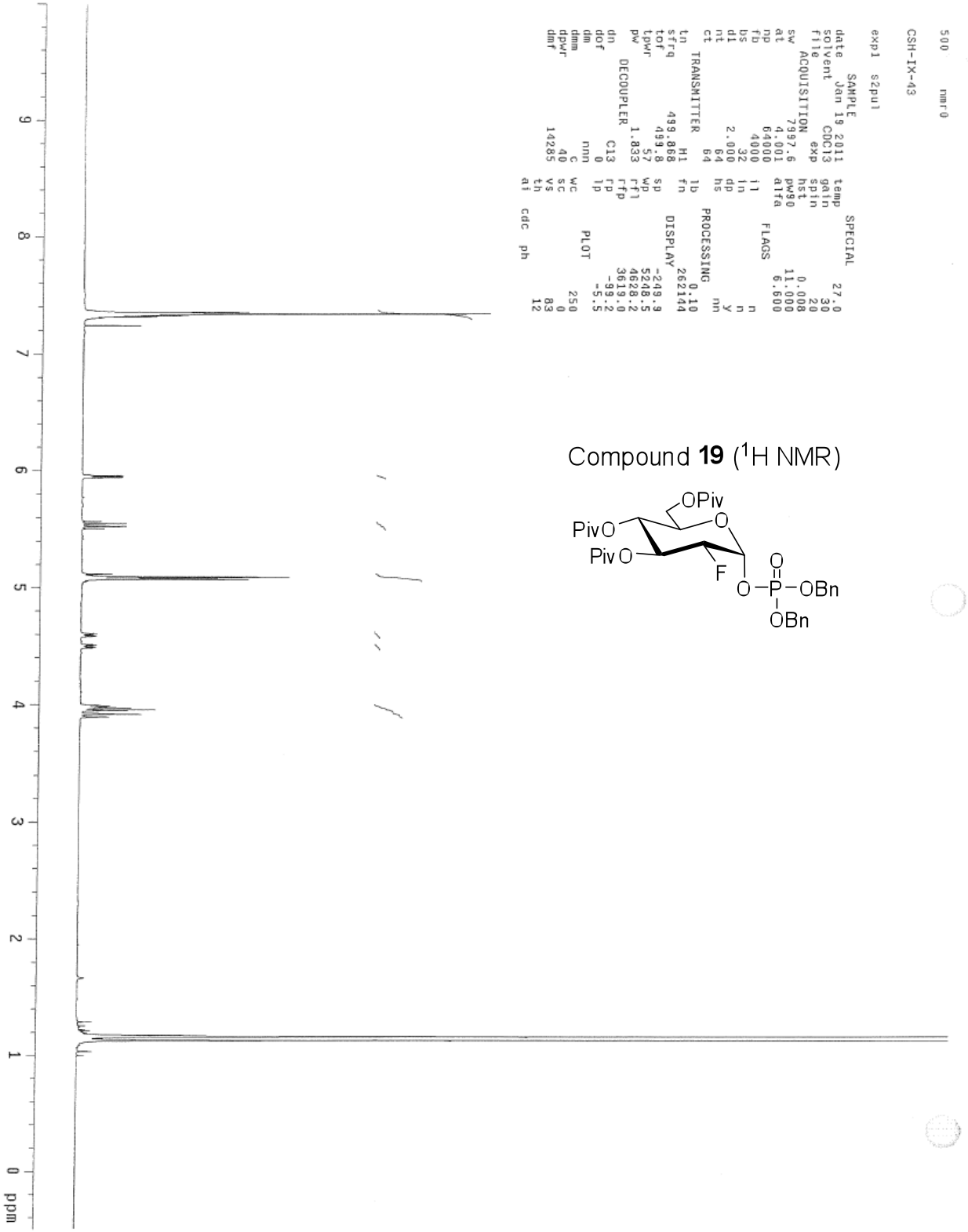


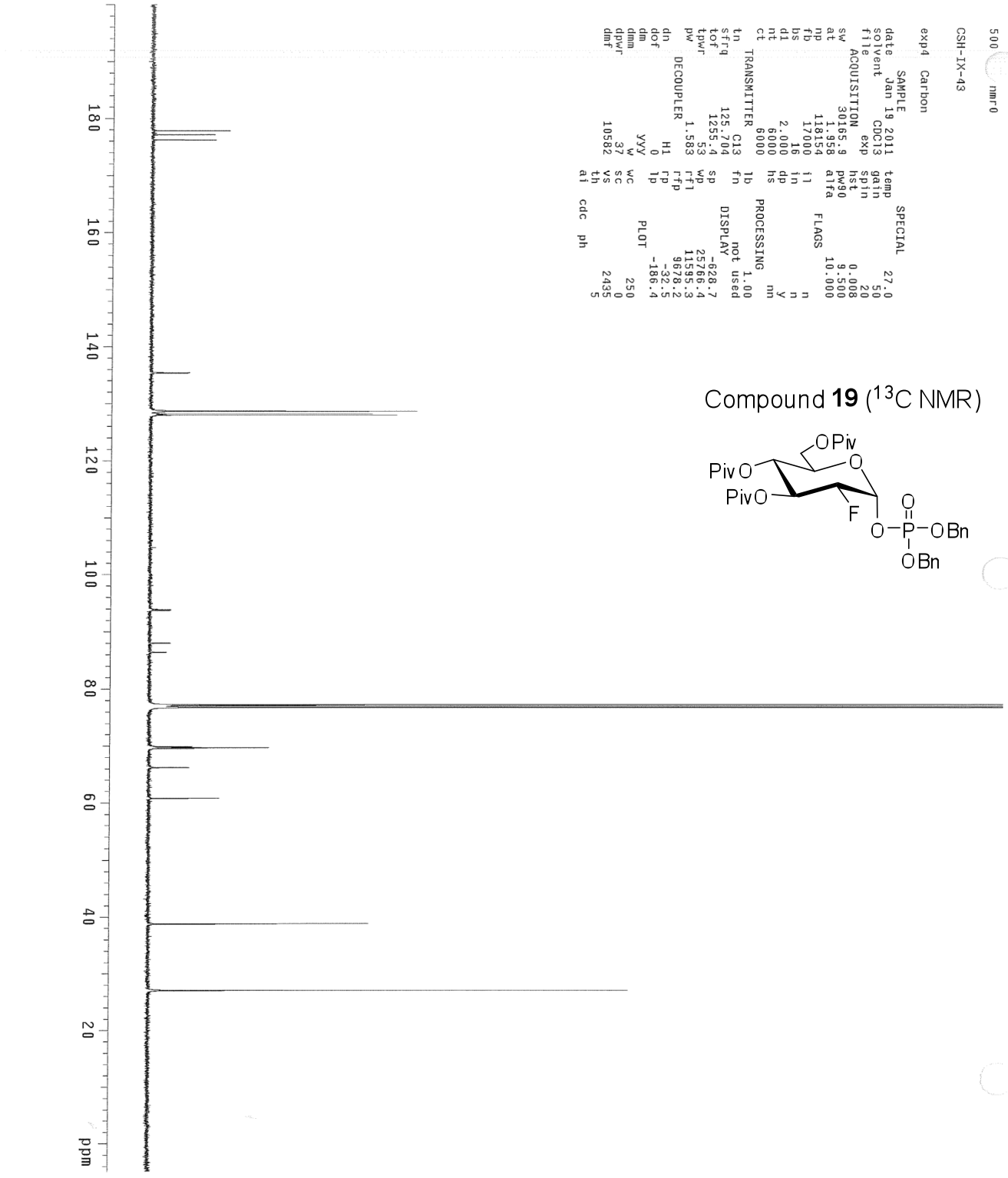
Figure S6. ESI (negative mode) MS data for the oxime adduct of UDP-4-KX, which is the peak with retention time of 35 min in HPLC trace.

S4. References

1. J. Sambrook, D. W. Russell, *Molecular Cloning: A laboratory manual* (Cold Spring Harbor Laboratory Press, New York, ed. 3rd edition, 2001).
2. M. Molhoj, R. Verma and W. D. Reiter, *Plant J*., 2003, **35**, 693.
3. Z. Tao, P. Gao, D. W. Hofmann, H.-w. Liu, *Biochemistry,* 2008*,* **47**, 5804.
4. S. P. Vincent, M. D. Burkart, C-Y. Tsai, Z. Zhang and C.-w. Wong, *J. Org. Chem.*, 1999, **64**, 5264.

S5. Spectroscopic data

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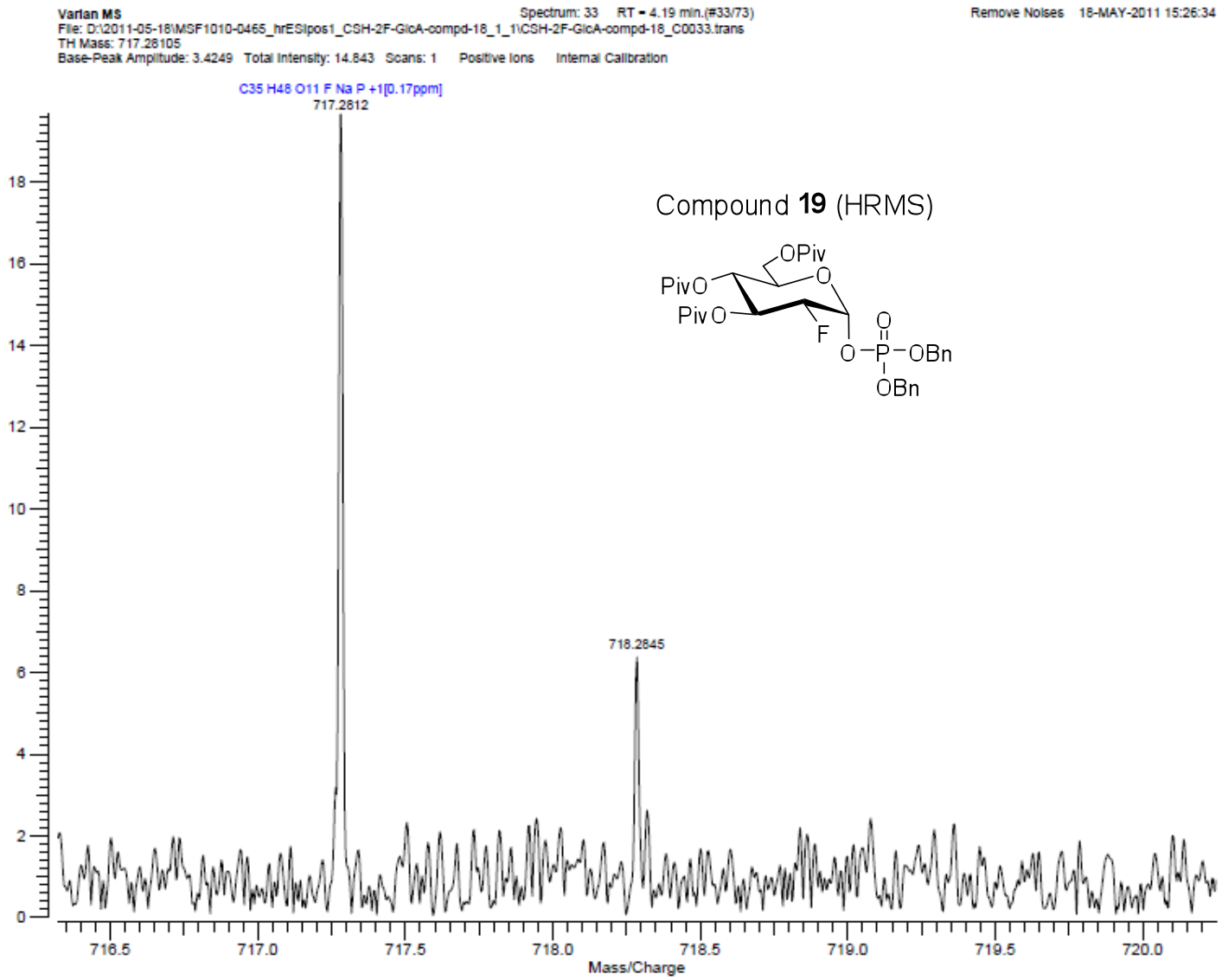
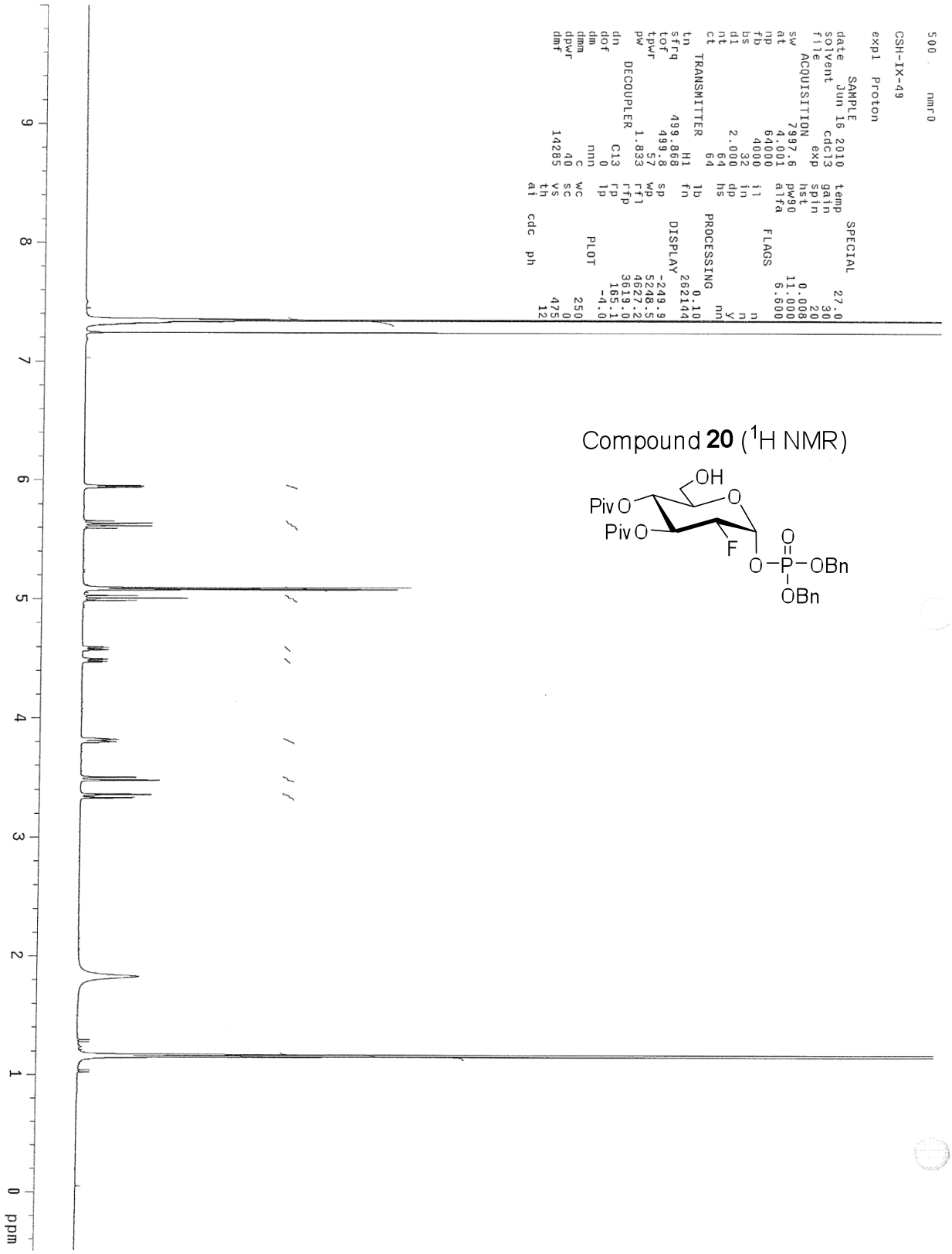
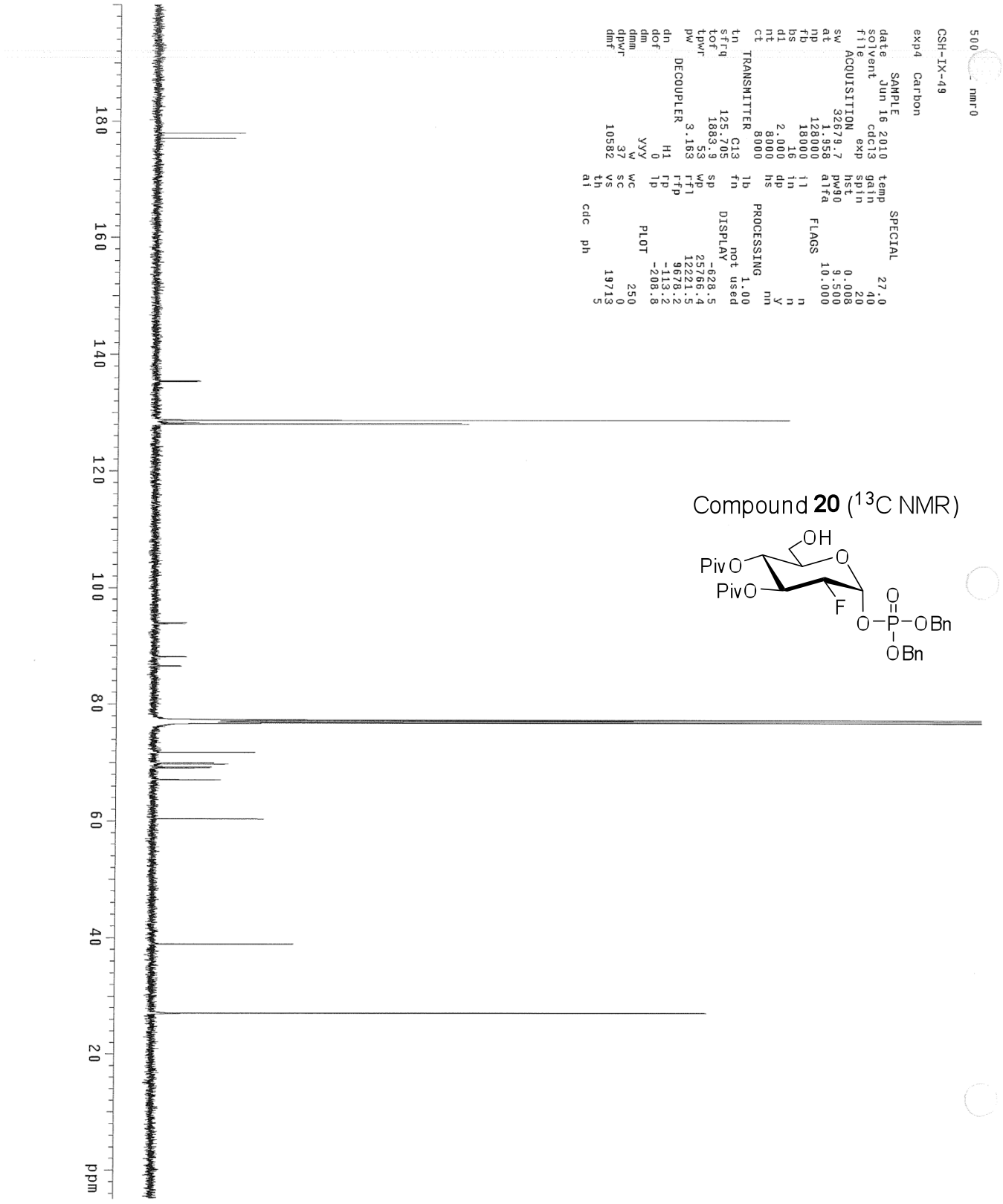
20
ppm

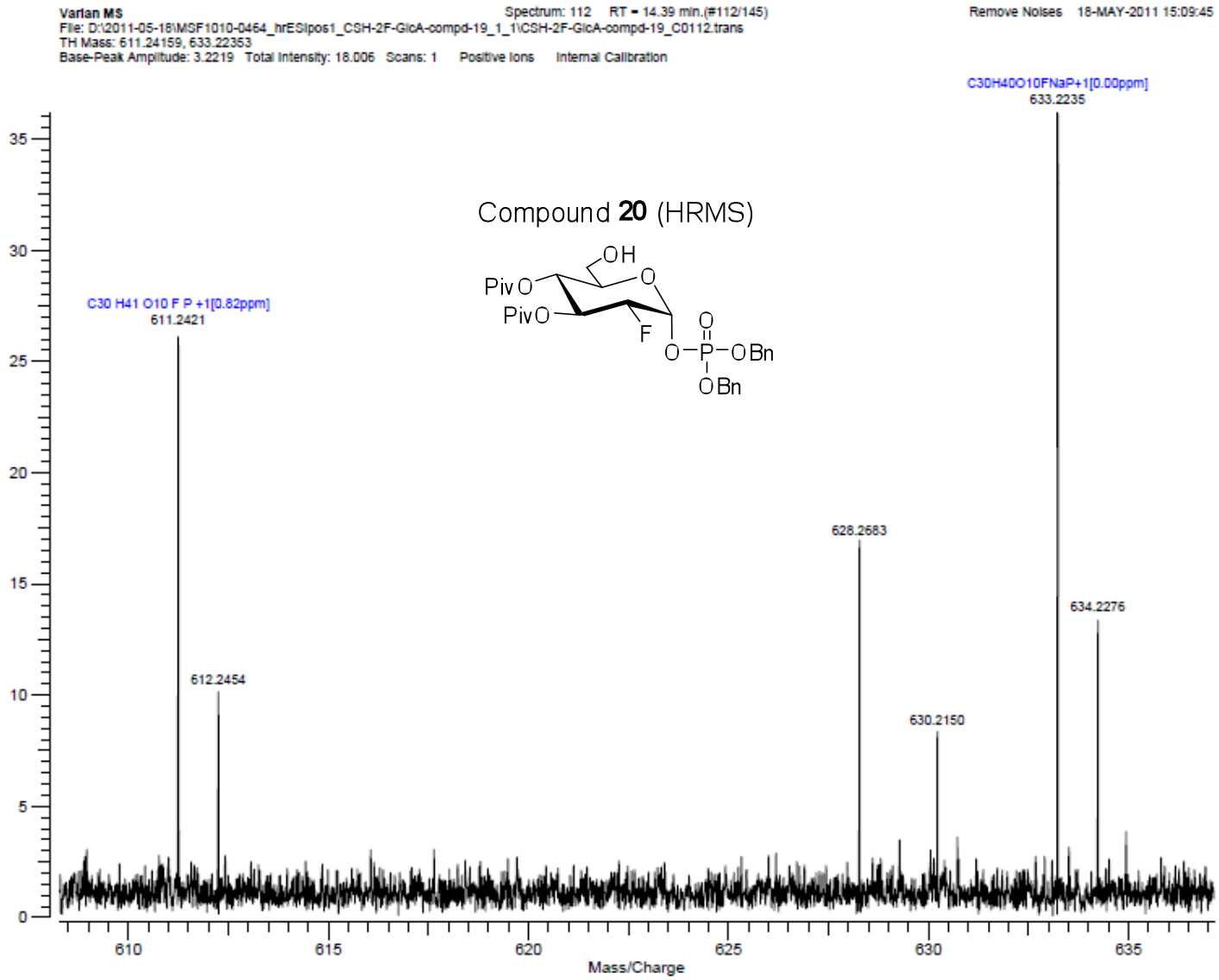


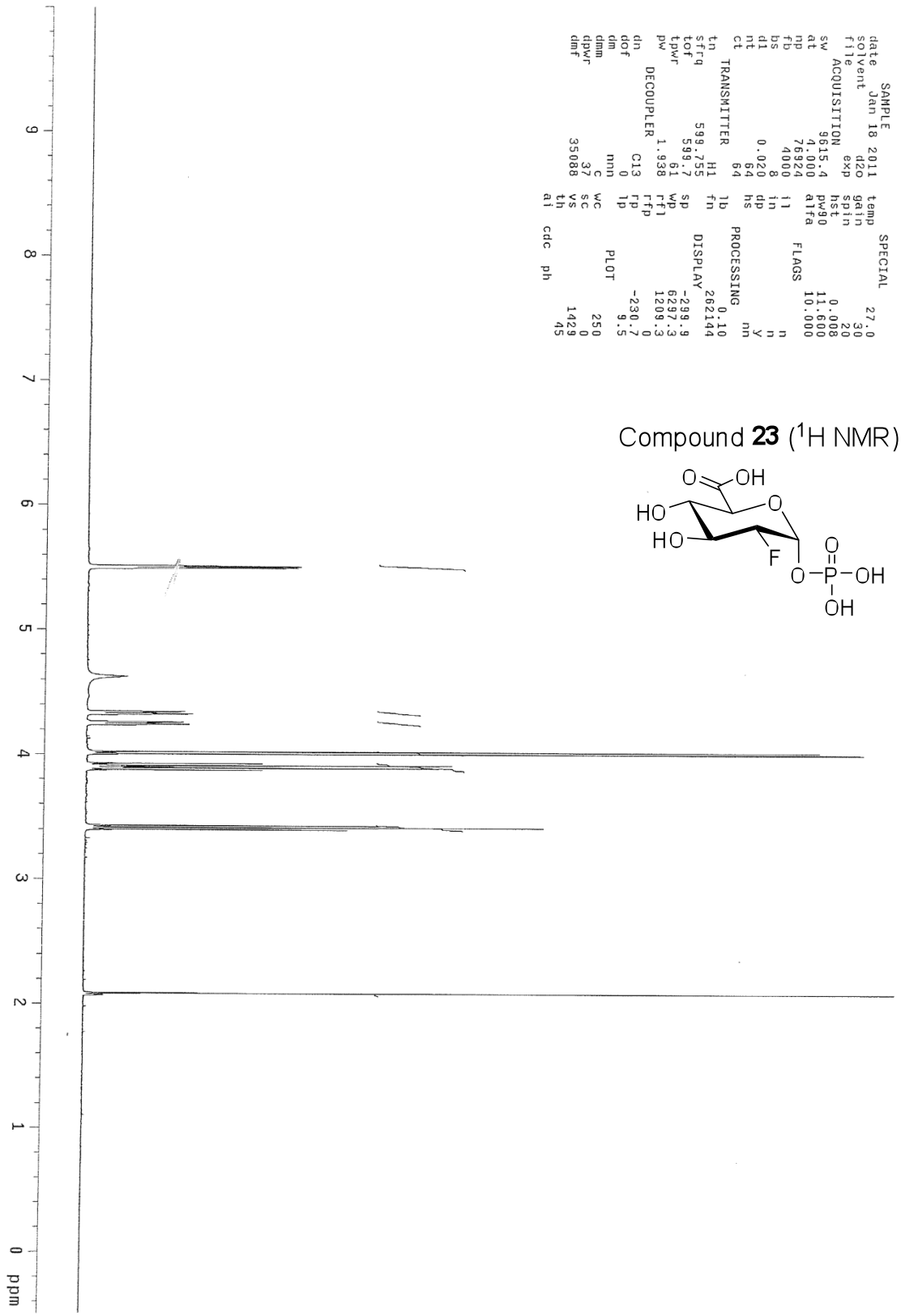


Compound **20** (^{1}H NMR)

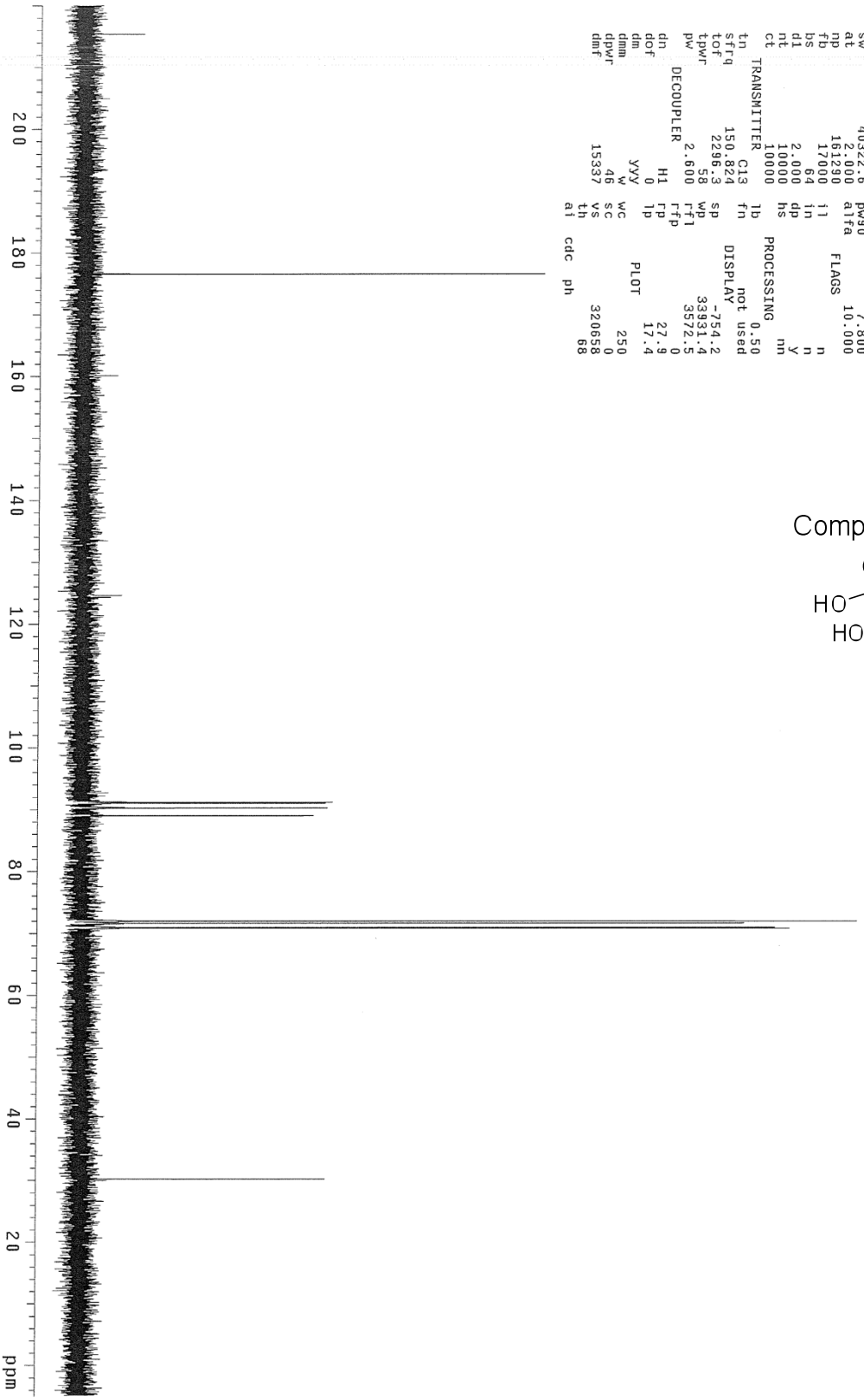


Compound **20** (^{13}C NMR)

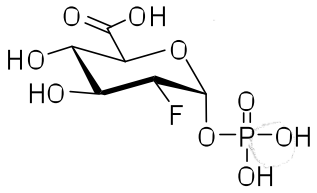

Varian MS
Spectrum: 112 RT = 14.39 min.(#112/145)
Remove Noises 18-MAY-2011 15:09:45
File: D:\2011-05-18\MSF1010-0464_hrESIpos1_CSH-2F-GlcA-compd-19_1_1\CSH-2F-GlcA-compd-19_C0112.trans
TH Mass: 611.24159, 633.22353
Base-Peak Amplitude: 3.2219 Total Intensity: 18.006 Scans: 1 Positive Ions Internal Calibration
C30H40O10FNaP+1[0.00ppm]
633.2235
Compound 20 (HRMS)
OH
O
Piv O
Piv O
F
O
O P O Bn
O Bn
C30 H41 O10 F P +1[0.82ppm]
611.2421
628.2683
634.2276
612.2454
630.2150
35
30
25
20
15
10
5
0
610
615
620
625
630
635
Mass/Charge

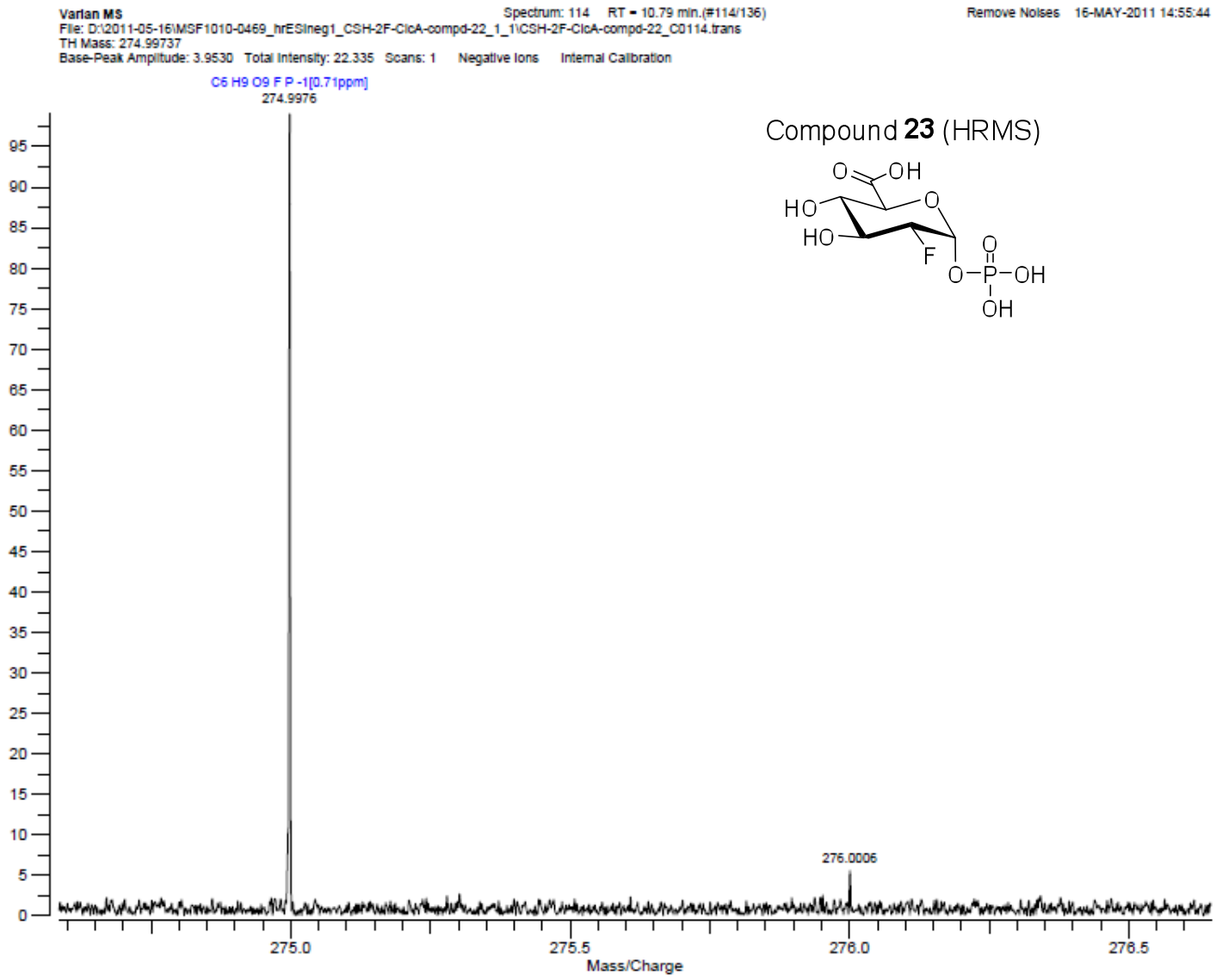


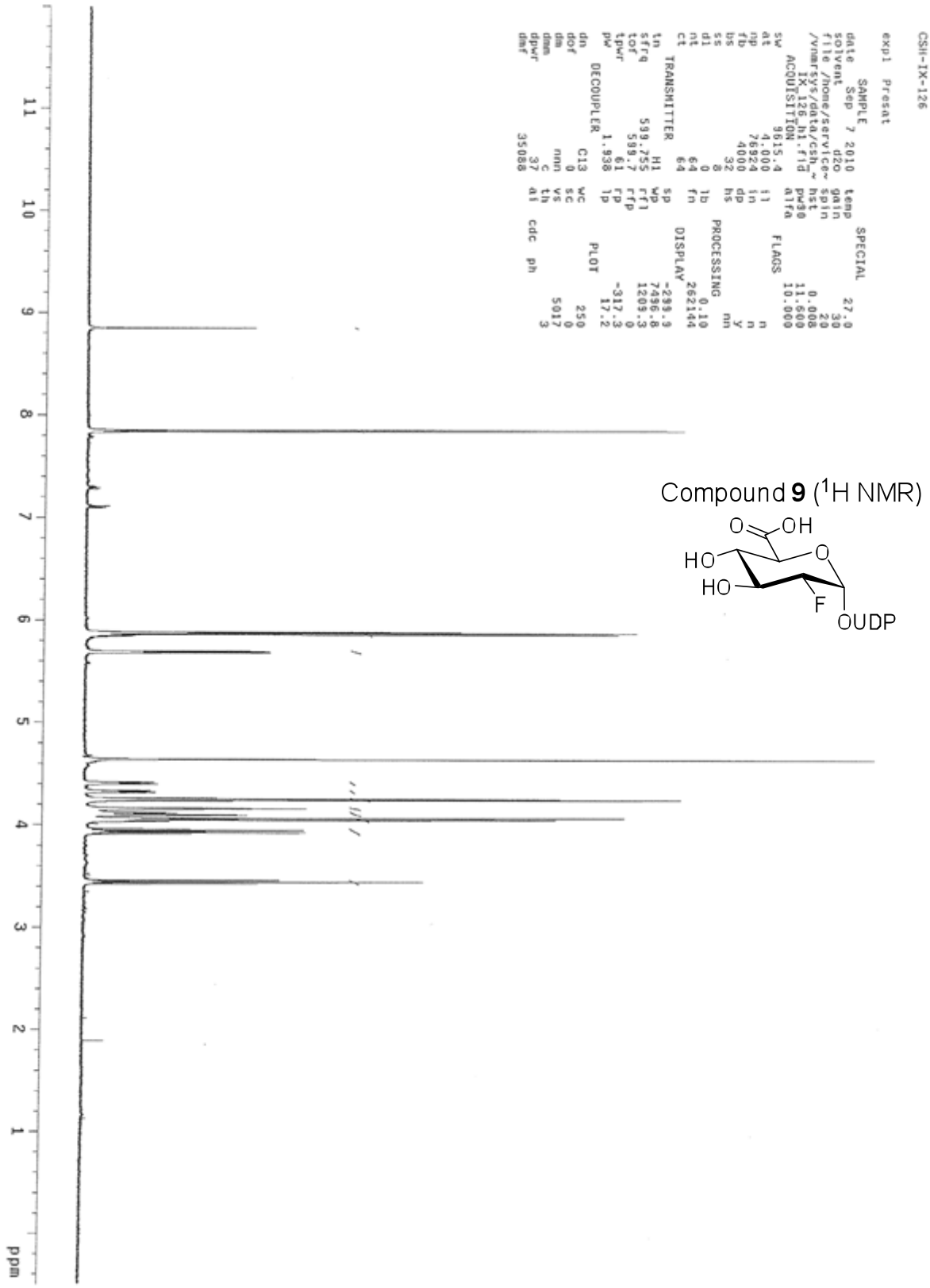
Compound **23** (^{1}H NMR)



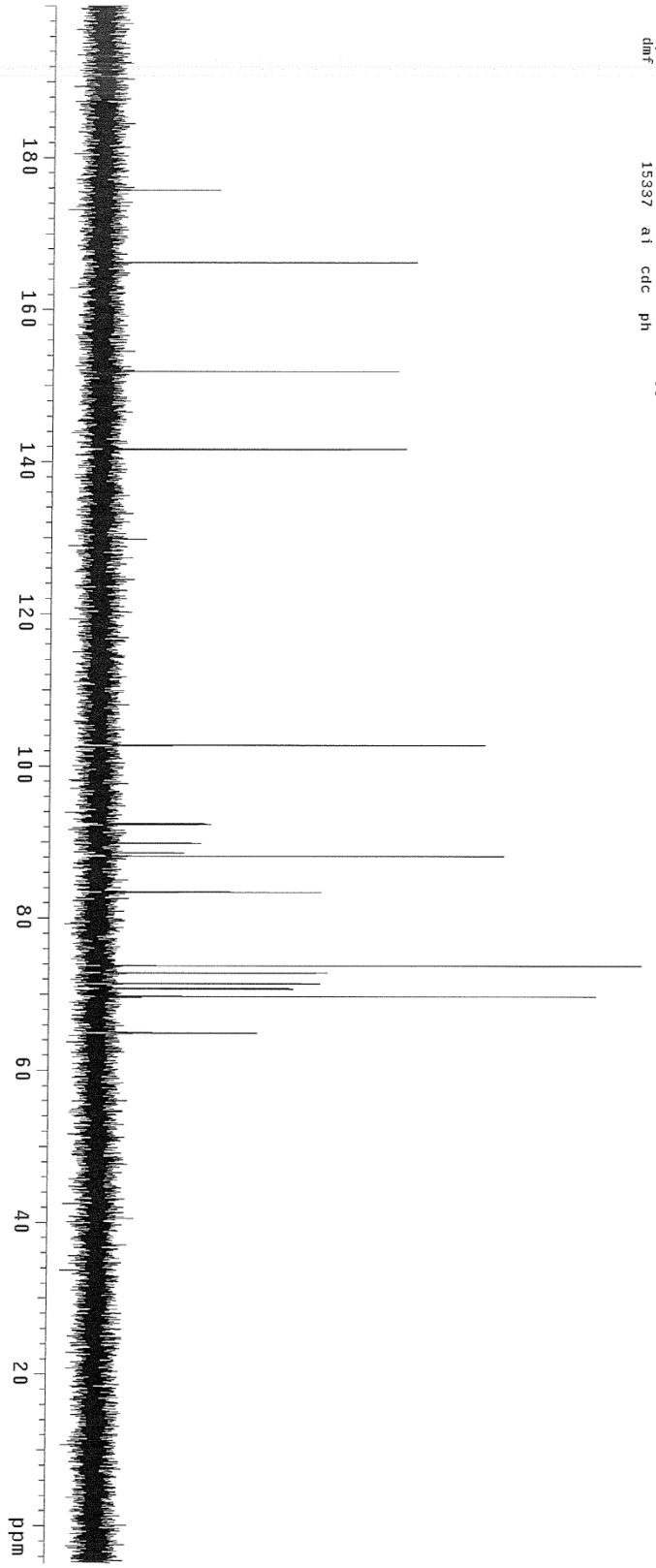
Compound **23** (^{13}C NMR)




Varian MS
Spectrum: 114 RT = 10.79 min.(#114/136)
Remove Noises 16-MAY-2011 14:55:44
File: D:\2011-05-16\MSF1010-0469_hrESIneg1_CSH-2F-ClcA-compd-22_1_1\CSH-2F-ClcA-compd-22_C0114.trans
TH Mass: 274.99737
Base-Peak Amplitude: 3.9530 Total Intensity: 22.335 Scans: 1 Negative Ions Internal Calibration
C6 H9 O9 F P -1[0.71ppm]
274.9976
Compound 23 (HRMS)
276.0006
Mass/Charge

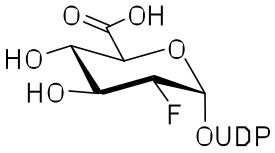


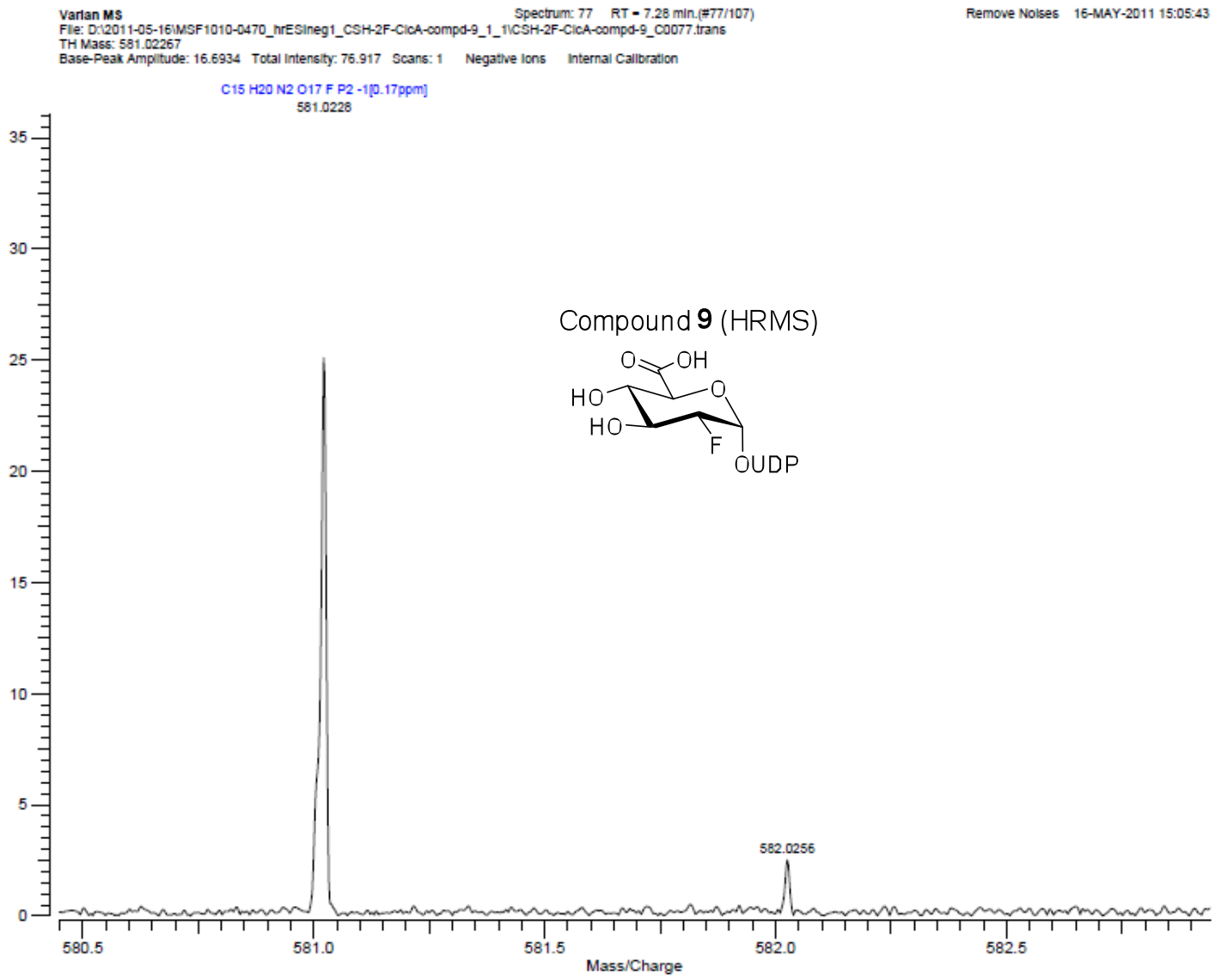
Compound **9** (^{1}H NMR)




```
600 MHz nmrox

CSH-IX-126

exp4  Carbon

                SAMPLE                          SPECIAL
date    Sep  7 2010    temp                      27.0
solvent             d2o gain                      40
file /home/service~     spin                      20
/vnmrsys/data/csh_~     hst                    0.008
    IX_126_c13.fid      pw90                   7.800
       ACQUISITION      alfa                  10.000
sw          40322.6                FLAGS
at              2.000   il                        n
np            161290    in                        n
fb            170000    dp                        y
bs                64    hs                       nn
d1             2.000              PROCESSING
nt              8000    lb                    0.50
ct              8000    fn                  262144
   TRANSMITTER                    DISPLAY
tn                C13   sp                  -754.2
sfrq          150.824   wp                 30915.4
tof            2296.3   rfl                3572.5
tpwr                58   rfp                     0
pw             2.600    rp                  112.3
            DECOUPLER   lp                   24.9
dn                 H1                PLOT
dof                 0   wc                   250
dm                yyy   sc                     0
dmm                 w   vs                107844
dpwr               46   th                    68
dmf             15337   ai   cdc  ph
```

Compound **9** (^{13}C NMR)




Varian MS
Spectrum: 77 RT = 7.28 min.(#77/107)
Remove Noises 16-MAY-2011 15:05:43
File: D:\2011-05-16\MSF1010-0470_hrESIneg1_CSH-2F-ClcA-compd-9_1_1\CSH-2F-ClcA-compd-9_C0077.trans
TH Mass: 581.02267
Base-Peak Amplitude: 16.6934 Total Intensity: 76.917 Scans: 1 Negative Ions Internal Calibration
C15 H20 N2 O17 F P2 -1[0.17ppm]
581.0228
Compound 9 (HRMS)
O
OH
HO
O
HO
F
OUDP
582.0256
35
30
25
20
15
10
5
0
580.5
581.0
581.5
582.0
582.5
Mass/Charge